

Bioconversion of Whey to Electrical Energy in a Biofuel Cell Using *Saccharomyces cerevisiae*

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Abstract: Biofuel cells represent a new technology for simultaneous use of waste materials and bioelectricity generation. In this study, electricity generation with whey degradation was investigated in a two compartment cell with and without mediators in the microbial fuel cell. *Saccharomyces cerevisiae* (PTCC 5269) was able to utilize the carbohydrate exist in the whey for generation of bioelectricity. The open circuit potential in absence of mediator was 500 mV at ambient temperature (25±2°C). The voltage was stably for duration of 2 days. Methylene blue and natural reds were used as potential mediators. The results showed that natural red was a few times more effective than methylene blue. Maximum power generation and current were 50 µW and 470 µA, respectively.

Key words: Whey • Bioelectricity • Biofuel cell • *Saccharomyces cerevisiae* • Mediator

INTRODUCTION

Microorganisms can be used as electron donor in biological fuel cell (BFC) for the conversion of organic matter into power [1,2]. Operation of biofuel cell is very similar to conventional fuel cell, except that they use biocatalyst such as enzyme, bacteria or other organism. BFC operates without presence of inorganic catalyst such as platinum, to catalyze the bioconversion of chemical energy in to electricity [3].

BFC generates electrical energy through the oxidation of biodegradable organic matter in the presence of either fermentative bacteria or enzyme under mild reaction conditions like ambient temperature and pressure [4]. The first observation of electrical current generated by bacteria is generally credited to Potter in 1911 (Potter 1911). In past decades, fuel cells became an interesting research field and work on BFC began to be reliable and attractive with more promising future [5]. Chaudhury and Lovley [6] used *Rhodospirillum rubrum* in BFC for power generation. It has been reported that the microorganism has generated electricity with an electron yield of 80%. The high electron recovery was also reported by Zhuwei *et al.* [3]. The power generation by microorganisms in BFCs is still very low. One of the suitable suggested techniques to resolve such problem is to store the electricity in rechargeable devices [3].

Most of BFCs use mediator molecule to speed up transfer of the generated electron to the electrode surface. Recently, mediatorless microbial fuel cells have been developed, the electrons were directly transferred to the electrodes [7]. Kim *et al.* [8,9] used *Proteus vulgaris* in BFC. Thionin was used as mediator in anode chamber. Park and Zeikus [10] have investigated the interactions between bacterial cultures and electron mediators. Effect of thionin and neutral red as mediator for oxidation and reduction of nicotinamide adenine dinucleotide (NAD⁺) was investigated. As the energy carrier biomolecules, NAD⁺ and NADH are the oxidized and reduced forms, respectively. Neutral red was found a better electron mediator than thionin for enhancing bioelectricity from glucose in a BFC using either *Escherichia coli* or *Actinobacillus succinogenes*. Although several types of mediators were used in BFC to enhance the electron transfer efficiency but they are generally too expensive to be used in commercial scale of BFC and also they can exhibit toxicity to living organism for long periods [11,12].

The soluble redox mediators have been added to BFCs for improvement of electron transfer. Redox mediators with potential enabling bacteria had sufficiently high turnover rate and high coulombic energy efficiency. Several researchers have developed advanced anode materials, by impregnation of anode with chemical catalysts. Park and Zeikus [10] used manganese modified kaolin electrodes with power output of 788 mW.m⁻².

The purpose of present research was to demonstrate BFCs for power generation using the treated whey. The performance of BFC for power generation with various geometric configurations and several process parameters were investigated. The main objective of the present study was to investigate the bioconversion of whey to electricity using *Saccharomyces cerevisiae* in the ode chamber of BFC.

MATERIALS AND METHODS

Whey was obtained from Gela Industry (Amol, Iran). Whole whey solution was uniformly acidified by acid solution (HCl, 5 N) at acidic pH (less than 4) to remove excessive proteins. The solution was autoclaved at 15psig, 121°C for 15 min, then cooled down to room temperature, centrifuged at 11,000 x g in sterilized tubes for 15 min to remove aggregated solids. The supernatant (whey supernatant), was refrigerated for 12 hours and it was used after adjusted pH to 7 by the concentrated NaOH solution (5M), as the major constituent of media for the growth of *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae PTCC 5269 was supplied by Iranian Research Organization for Science and Technology, Tehran, Iran. The microorganisms were grown in an anaerobic jar vessel. The medium prepared for seed culture consisted of glucose, yeast extract, NH₄Cl, NaH₂PO₄, MgSO₄ and MnSO₄: 10, 3, 0.2, 0.6, 0.2 and 0.05 g/l, respectively. The medium was sterilized, autoclaved at 121°C and 15psig for 20 min.

The treated whey was used as carbon source and the whey's carbohydrate was considered as lactose. The medium pH was initially adjusted to 6.5 and the inoculum was introduced then the culture was incubated at 30°C. The organism was fully grown for duration of 24 hours in 100 ml flux without any agitation. Samples were drawn in time interval of 4 h and substrate consumption was determined based on reduced sugar by DNS method [13]. Before analysis, liquid samples were filtered by a 0.45 µm syringe membrane (Sartorius Minisart). All chemicals and reagents used for the experiments were analytical grades and supplied by Merck (Germany). The pH meter, HANA 211(Romania) model glass-electrode was employed for measuring pH values in the aqueous phase.

DNS method was developed to detect and measure substrate consumption using colorimetric method [13] and cell growth was also monitored by optical density using spectrophotometer (Unico, USA).

SEM was used to show the surface of the membrane (Nafion 117) and electrodes. The sample coated with gold and observed with a (Phillips XL30, Holland) microscope. Finally, images of the samples were taken under SEM at magnifications of 5000, 10000 and 20000.

The fabricated cells in the laboratory scale were made of glass (Pyrex) material. The volume of each chamber (anode and cathode chambers) was 850 ml with working volume of 760 ml. The sample port was provided for the anode chamber, wire point inputs and inlet port. The selected electrodes in BFC were graphite in size of 40 × 70 × 2 mm. Proton exchange membrane (PEM; NAFION 117, Sigma–Aldrich) was used to separate the two compartments. The Nafion area separated the chambers was 3.79 cm². Nafion proton exchange membrane was subjected to a course of pretreatment to take off any impurities that was boiling the film for 1h in 3% H₂O₂, washed with deionized water, 0.5 M H₂SO₄ and then washed with deionized water. The anode and cathode compartments were filled by deionized water when the biological fuel cell was not in use to maintain membrane for good conductivity. Methylene blue and natural red were supplied by Merck (Germany). These chemicals with low concentration (100 µmol/l) were used as mediators in BFC. The schematic diagram and auxiliary equipment of the fabricated BFC cell is shown in Figure 1.

Analog digital data acquisition was fabricated to register data point in every 4s. The system had measurements for variable resistances which were imposed to the BFC. The current in BFC was recorded, dividing the obtained voltage by the defined resistance. Then, the system provides power calculation by multiplication of voltage and current. Also the online system demonstrates polarization graphs for power generation and BFC voltage with respect to current. The online system has the ability to operate automatically or manually. While it operates in auto-mode, the assembled relays are able to regulate automatically the resistances. Voltage of BFC was amplified and then data was transmitted to a microcontroller by an accurate analog to digital converter. The microcontroller also sends the primary data to a computer by serial connection. Also special function of MATLAB software (7.4, 2007a) was used to store and synchronically display the obtained data.

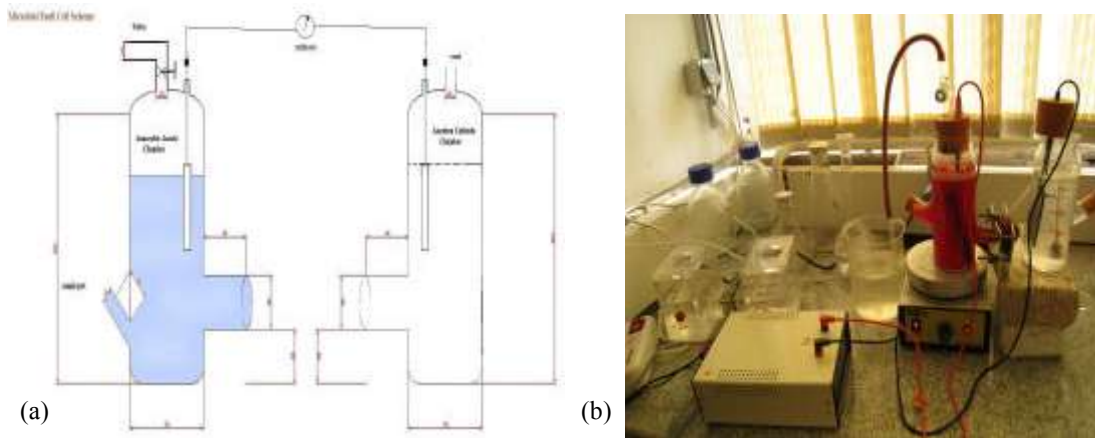


Fig. 1: Fabricated BFC (a) Schematic drawing (b) BFC picture with the auxiliary equipments

RESULTS AND DISCUSSION

The fabricated BFC was used for power generation using whey as carbon source. The abundant and cheap feed source of chemical energy was used for the purpose of power production. The fast growing microorganism was implemented for power generation [14]. The entire experiments were classified as electrodes with and without mediators. The role of mediators as electron promoters was investigated.

Figure 2 shows open circuit voltage recorded for the BFC in period of 48 hours. Initially, the voltage was less than 200 mV and then gradually increased. After 8 hours of operation, OCV reached to a maximum value of 500 mV. The OCV was quite stable for the whole 2 days. The instability was due to lactose depletion in the anode compartments after 2 days.

Figure 3 depicts polarization curve without the presence of any electron mediator. The presented data shows power and voltage with respect to current generated in the BFC. Maximum power generation and current were 1.43 μ W and 11.5 μ A, respectively.

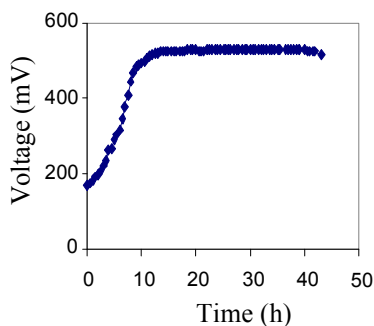


Fig. 2: Open Circuit Voltage (OCV) of the BFC

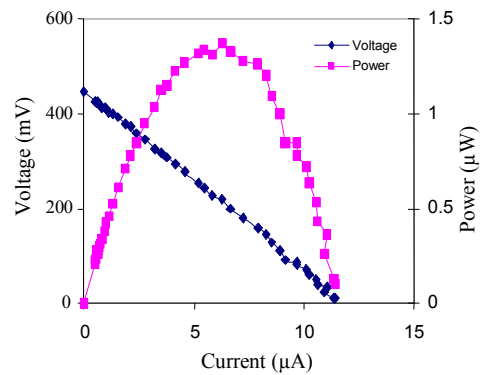


Fig. 3: Polarization curve without the presence of any electron mediator

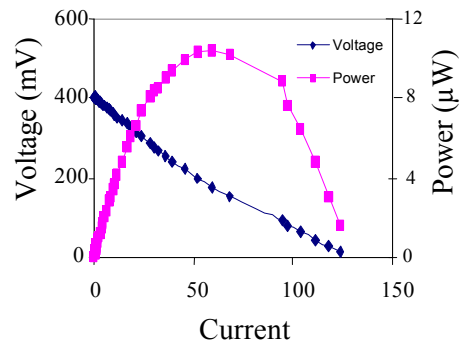


Fig. 4: Polarization curve presence of Methylene Blue as electron mediator

Mediators are normally used to enhance the performance of BCF [15,16]. Electron transfer in anode compartment was promoted by two types of mediators in the fabricated BFC.

Methylene blue and natural red were selected as a mediator in BFC with concentration of 100 μ mol/l. The mediator in BFC had increased the power

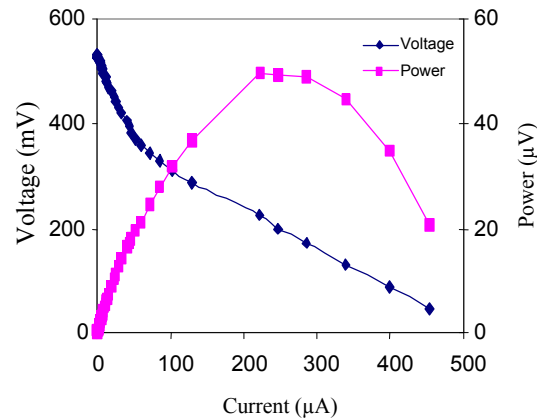


Fig. 5: Polarization curve presence of Natural Red as mediator

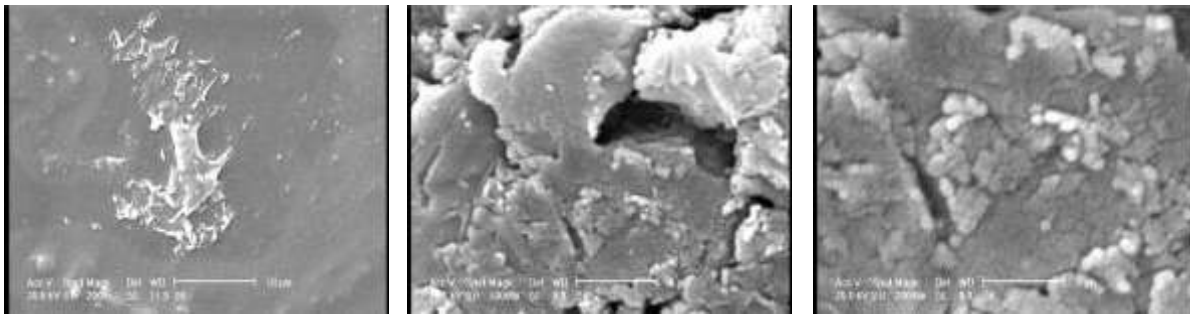


Fig. 6: Scanning electronic microscopy of the outer surface of the used membrane (Nafion 117) (a) Nafion, magnification of 5000 (b & c) Graphite electrode, magnification of 10000 and 20000

generation and cell current. Methylene blue increased the power and current to 11.3 μW and 120 μA , respectively (Figure 4). Figure 5 presents a few fold increase in power and current production by the use of natural red as a suitable mediator. The power generation and current production in presence of natural red was the highest in compare with methylene blue. Maximum power generation was 50 μW while the current was boosted to the highest value of 47 μA .

Figure 6a shows Scanning electronic microscopy technique has been widely applied to provide surface and morphological information. The morphology of used membrane (Nafion 117) was examined with scanning electronic microscope (SEM) as one of the essential components in the BFC. A piece of the Nafion (1 \times 1cm) was analyzed by scanning electronic microscope. In addition the images of the surface characteristic of the graphite plate electrode with two different magnifications were obtained successfully by SEM. Graphite electrode

was removed at the end of experiment and cut into pieces of about 1 \times 1cm for SEM analysis. Figs. 6b and 6c show the outer surface of the graphite electrode with magnification of 10 000 and 20 000, respectively. These images demonstrated microorganism grown on the graphite surface. Some clusters of microorganisms were observed in several places on anode.

CONCLUSIONS

Bioelectricity generation was successfully adapted in the BFC. Whey was utilized as carbon source for the electrical energy *Saccharomyces cerevisiae*. The BFC performance was enhanced by the use of chemical mediator. Natural red served as suitable mediator and enhanced the electrical energy by 5 folds. Also the current production was increased to 470 μA . Based on the SEM characteristics, membrane must be change or regenerated for the further applications.

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